SECTION 11

SAMPLE HANDLING AND CUSTODY REQUIREMENTS

11.1 SAMPLE HANDLING

11.1.1 Sample handling requirements vary with the assemblage being studied in the survey. For most biological assessments, the minimum sample size needed to fulfill the data quality objective for representativeness should be incorporated into the sampling design so that sampling produces minimal environmental impact. For those samples that will be analyzed in the laboratory, the organisms are sacrificed and field preservation, labelling and transport protocols must be followed. For many fish surveys, experienced fish biologists are proficient in field identifications and thus most specimens are returned to the water following identification and enumeration. Exceptions are juveniles, hybrids, and difficult-to-identify species. Also, if temporary crews are used, samples of all collections should be verified. Voucher specimens are appropriate for all species and should be stored in fish museums or universities whenever possible. For a review of fish methods, readers should refer to USEPA (1993a), Meador et al. (1993), or Ohio EPA (1989). Most of the following information is appropriate for those types of samples that are returned to laboratories for processing and identification (benthic invertebrates, phytoplankton, and periphyton).

11.1.2 All activities categorized under sample handling should be documented (as SOPs) and followed closely to prevent or minimize the introduction of error. Consistency should be the rule in all of the following activities:

- field preservation
- labelling
- storing or transportation.

11.1.3 The following information associated with each sample should be identified:

- exact location and ambient conditions associated with sample collection should be maintained in field notebooks, field collection sheets, or PDRs; possession and analysis logs should be maintained in the laboratory;

- chain-of-custody forms, sample preservation, if any, and dates and times of sample transfer and analysis;

- procedures for transferring and maintaining custody of samples.

11.1.4 Certain sampling protocols (e.g., the Tier 2 protocols of Plafkin et al. [1989] and most fish sampling) involve sorting, identification, and enumeration of specimens in the field. When benthic macroinvertebrate and fish samples are field-identified, the field data sheets become the item of custody as do any preserved specimens used for taxonomic verification. All header information should be completely filled out and copies of all sheets distributed. As specimens are laboratory-identified, they can be preserved, archived as vouchers, and placed in a repository. Location of the repository and a record of the specimen preservation is entered into a log book.

11.2 SAMPLE CUSTODY

11.2.1 The primary objective of the chain-of-custody procedure is to create a written record that can be used to trace the possession of the sample from the moment of collection through the entire data analysis. Field crews as well as laboratory personnel should follow written chain-of-custody procedures for collecting, transferring, storing, analyzing, and disposing samples. Sample custody procedures are important to ensure the integrity of the samples whether for legal or other purposes. Explicit procedures must be followed to maintain the documentation. An example chain-of-custody form is presented in Figure 11-1; separate forms should be filled out for each sample if the samples are likely to become separated. Notations should be entered in the logbook regarding the condition of the samples.

All sample labels, as well as the chain-of-custody form, should contain the following information at a minimum:

- ID or log number (can be same as sample no.)
- Location - state, county, approximate distance from nearest town, name of waterbody being sampled
- Date - date of sample collection
- Time - time of sample collection
- Sampled By - initials of personnel collecting the sample
- Type of Sample - e.g., benthos, periphyton
- Preservative - e.g., formalin, 95 percent ethanol, Lugol's solution
- Station - numbers or letters to designate station location
- Sampling Gear - e.g., kicknet, seine, eyedropper.

11.2.2 Samples from which courtbound data are to be derived are kept in sample storage areas of the laboratory where access is limited to laboratory personnel and controlled by locked doors; treating all samples as if they were courtbound decreases the likelihood of mishandling actual courtbound samples. The samples are routinely retained at the laboratory as required by the project after the data have been forwarded to the appropriate person(s) so that any analytical problems

Project Manager or Contact: Address/Phone:	# samples	# jars		Sample Type							CHAIN-OF-CUSTODY RECORD Agency Name: Address:	
Project Number: Project Name:												
Page of Sample Location:												
Date	Time	Sample Station									Gear Method	Log Number
Sampled by: *(signature)*	Date/Time:	Relinquished by: *(signature)*	Date/Time:	Received by: *(signature)*	Date/Time:							
Received by: *(signature)*	Date/Time:	Received by: *(signature)*	Date/Time:	Received by: *(signature)*	Date/Time:							

FIGURE 11-1. Chain-of-custody record.

can be addressed. The samples are discarded at the end of a specified time period (see Table 12-1); 1 to 5 years may be appropriate, depending on project requirements. Long term preservation methods of biological samples can be found in Klemm et al. (1990). A sample evidence file should be maintained which includes copies or original laboratory bench sheets, field notes, chain-of-custody forms, logbooks, sample location and project information, and final report. The location and responsible agency of the evidence file should be named in the project plan.

11.2.3 Specific tasks/conditions for sample storage may include the following:

- Samples will be stored in a secure area.
- The secure area will be designed to comply with the storage method(s) defined in the contract (i.e., fireproof, ventilated, etc.).
- The samples will be removed from the shipping container and stored in their original containers unless damaged.
- "Damaged and unusable samples" (for example, a sample container that broke and part or all of the sample was not recoverable) will be disposed of in an appropriate manner and disposal will be documented.
- "Damaged and usable samples" (for example, a sample container that broke in such a way as to salvage *all* organisms) will be documented and transferred to a new container, if possible and necessary. The field leader and the Project Manager will be notified immediately of any damaged or disposed samples.
- The storage area will be kept secure at all times. The sample custodian will control access to the storage area. Duplicate keys for locked storage areas should be maintained only by the appropriate personnel.
- Whenever samples are removed from storage, this removal will be documented; all transfers of samples can be documented on internal chain-of-custody records.
- Samples, reference, and voucher specimens (section 12.7) will be stored after completion of analysis in accordance with the contract or until instructed otherwise by the Project Manager.

- The location of stored reference or voucher specimens will be recorded.

- Reference or voucher specimens will not be stored with samples.

- The sample storage area will be described.

SECTION 12

ANALYTICAL METHODS REQUIREMENTS

12.1 Methods of sample and data analyses should be well-documented. These methods must be appropriate for all parameters and should be USEPA-approved or otherwise validated/published standard methods.

12.2 For USEPA-approved or standard methods, pertinent literature should be referenced. Pertinent literature would include appropriate validation data for the methods to be used.

12.3 For non-standard, state developed, or modified methods (Caton 1991), detailed SOPs should be provided which include methods for all sample preparation, picking and sorting, and identification procedures.

12.4 BIOLOGICAL SAMPLE LABORATORY PROCESSING

12.4.1 Biological sample laboratory processing generally falls into two broad divisions. The initial or primary sample processing may include sorting, subsampling, and re-sorting checks. Secondary or final phase processing may include taxonomic identification and verification procedures, tabulation, enumeration, and measurements. The secondary phase might also include calculation of metrics or indices. An example of a macroinvertebrate data sheet is presented in Figure 12-1.

12.5 PRIMARY PHASE SAMPLE PROCESSING

12.5.1 Subsampling - In biomonitoring programs where resource limitations restrict expendable sampling and analytical effort, subsampling is recommended as a cost-effective and valid procedure for (a) selecting a representative estimate of the total sample collected and (b) standardizing the level of effort expended on each sample. Subsampling methods vary according to the assemblage. For example, methods may include procedures for cleaning diatom strewn mounts, and establishing counting transects on coverslip (Bahls 1992). Caton (1991) has developed a gridded screen technique for increased objectivity in field or laboratory subsampling of benthic macroinvertebrates. As subsampling methods are developed, every attempt should be made to reduce bias. SOPs should, therefore, be developed to standardize the unit of effort and to eliminate subsampler subjectivity and errors in sorting and picking. Subsampling error should be quantified depending on the type and volume of the subsample.

12.5.2 Sorting macroinvertebrates includes rough segregation of individuals within a sample or subsample by some predetermined taxonomic grouping into pre-

labelled containers. Sorters should be trained so that they can identify the organisms from the surrounding debris; QC checks on new sorters should be frequent until it is clear that the sorter knows what he or she is looking for. Subsequent sorting results in containers of finer taxonomic groupings. For algae samples, this step may include estimates of relative abundance of non-diatom (soft-bodied) algal taxa determined by cells per field of view of a composite wet mount (Bahls 1992).

12.5.3 Sorting Checks (post-"primary sorting") - A portion of sample residues must be re-checked by intralaboratory QC personnel for missed specimens (under-recovery). Re-sorting checks can be used to measure repeatability. A portion of sample residues may also be re-checked by separate laboratories for interlaboratory QC.

12.6 SECONDARY PHASE SAMPLE PROCESSING

12.6.1 Taxonomic Identifications, Verification Procedures - Training, experience, and possession of proper laboratory equipment and taxonomic literature are crucial factors affecting the quality of identification activities. Abbreviations commonly used in documentation (e.g., for scientific names) should be standardized and defined in the data pack to decrease data manipulation errors. A general guide is that specimens should be identified to the lowest possible taxonomic level using the most current literature available. Some parameters or other analytical techniques, however, may only require identification to the ordinal, familial, or generic level (Plafkin et al. 1989; Ohio EPA 1987). An argument against such an approach is that sensitivity and tolerance information is more accurate at the species level of identification.

All questionable taxonomic identifications should have a post-determined level of uncertainty identified. For instance, define a scale of uncertainty (e.g., 1-5 where 1 is most certain and 5 is least certain) for each identification and specify reasons for any uncertain identification (e.g., missing gills, headless specimen, etc.). Define the criteria for assigning tolerance values to uncertain identifications. For example, if the generic level of identification is questionable, determine an average tolerance value for the family level. For those taxa that are in good condition and easily identified by the taxonomist, the rating can either be noted as 1 (certain) or left blank.

12.6.2 Verification should be done in one of two ways: Comparison with a pre-established reference or research specimen collection can yield rapid and

Benthic Macroinvertebrates Laboratory Bench Sheet

Site/Project# Sample No.

Location Sampling Station

Type of Sample (Gear) Subsample: Total 100 200 300 Other

Taxonomist Date Sampled

Sorter

Enter Family and/or Genus and Species name on blank line. A = Adult I = Immature

Organisms	No	A	I	TCR	Organisms	No	A	I	TCR
Diptera					Heteroptera				
Chironomidae									
					Coleoptera				
Other									
					Neuroptera and Megaloptera				
Trichoptera									
					Crustacea				
					Oligochaeta				
Plecoptera									
Ephemeroptera					Hirudinea				
					Bivalvia				
					Gastropoda				
Odonata									
					Other				

Taxonomic certainty rating (TCR) 1-5: 1 = most certain, 5 = least certain. If rating is 3-5, give reason (e.g., missing gills).

Total No. Organisms_____ Total No. Taxa_____

FIGURE 12-1 Macroinvertebrate laboratory bench sheet.

accurate results. A reference collection is defined as a set of biological specimens, each representing some taxonomic level and not necessarily limited to specific projects or activities. Reference collections should have expert confirmation of each taxon.

Reference collections are used for verifying identifications of subsequent samples. One potential problem with this approach may be the previous misidentification of the reference specimens. An approach most likely entailing the least uncertainty is to send samples to taxonomic experts familiar with the group in question for confirmation (Borror et al. 1989). Detailed documentation of independent taxonomic verification by recognized experts should be provided along with address and telephone number. Potential problems might result by establishing a set of contacts among recognized experts in various groups of organisms. The taxonomist should *always* be contacted by telephone or correspondence prior to sending specimens. Just as important is the receipt of advice on proper methods for preserving, packing, and shipping samples to them. Damaged specimens are often useless and impossible to identify; thus, careful preservation and packing is essential.

12.7 VOUCHER COLLECTION

12.7.1 The true data of a project are the actual specimens collected in a survey for that project. Following identification and enumeration, these specimens should be maintained in a voucher collection. Voucher collections can be maintained any specified length of time for the project. For instance, if space is a critical issue, the voucher collection can be disposed of after the data have been reviewed and the report finalized.

12.7.2 Voucher collections may sometimes serve as reference collections but usually not vice-versa. This is primarily because reference collections are arranged/curated based on taxonomic and/or phylogenetic order and are not usually associated with particular projects or specific waterbodies (although that information will be included with label data). If there are ever questions regarding the accuracy of taxonomic identification that have been used in parameter calculation and reporting, referral to the voucher collection will be an initial step taken in resolution. Also, a complete list of taxonomic references used should be compiled for each project such as is found in USEPA (1990a). A comparison of various attributes of reference and voucher collections is presented in Table 12-1.

TABLE 12-1 Comparison of reference and voucher collections.

CONSIDERATIONS	REFERENCE	VOUCHER
Usual Curatorial Arrangement	Taxonomic and/or phylogenetic	By project or sample lot
Taxonomic Verification	By expert	By expert, or comparison to reference collection
Number of individual organisms by designated taxon	At least one, but several may be included (or added over time) to illustrate sexual dimorphisms or other morphological variability (including deformities) as well as to document geographic distribution	If full number of individuals enumerated from sample and used in data calculation is archived, it serves as the *sample voucher;* if a selected number of individuals from a sample (e. g., 10-20 out of 100 total) that represent an identifier's concept of a particular taxon, the specimens serve as the *taxonomic voucher*
Slide-Mounted Specimens	Permanent	Permanent or temporary
Required Storage Space	Large, but would tend to increase only as fast as additional taxa are incorporated; will be somewhat dependent on geographic area of responsibility	Could be very large, and could continue to grow at rapid pace if there are vouchers retained from all samples from all projects; particularly a problem with archiving of fish samples, progressively less so with benthos and periphyton. Fish vouchers should be deposited in a state or regional fish museum or university, where they are also useful for systematic and biogeographical research by others.
Can serve as reference collection?	----------	Sometimes, if curated in taxonomic arrangement within each project or sample lot; if project-oriented samples are segregated by taxon and distributed in collection among appropriate taxonomic groupings
Can serve as voucher collection?	Usually not, reference collections don't contain the totality of a sample; if they do, it *could* (but, not necessarily) take considerable effort to reassemble the sample; also, will not normally contain representative specimens from all project samples thus limiting its utility as a voucher collection	----------

TABLE 12-1 Continued.

CONSIDERATIONS	REFERENCE	VOUCHER
Duration of Maintenance	Permanent, ongoing	As required by contract or project specifications (e. g., project terms may specify maintenance of vouchers for a period of 5 years following final report approval after which they may be discarded); may be permanent; after this, and if not already done, voucher specimens may be incorporated into the reference collection
Major function and use	Taxonomic verification of future identifications	Data verification for specific projects